Thiamine deficiency in amyotrophic lateral sclerosis

Running title: Thiamine deficiency in ALS

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Abstract

Objective: Hypovitaminoses by virtue of undernourishment still represent clinical relevant diseases with a broad spectrum of symptoms. In this article, we discuss two patients with amyotrophic lateral sclerosis (ALS/MND) exhibiting the pattern of acute Wernicke Encephalopathy at autopsy. Therefore, we were interested in the general prevalence of thiamine (vitamin B1) deficiency in ALS/MND patients.

Method: We tested 122 ALS/MND patients without respective clinical symptoms for thiamine deficiency (transketolase blood levels and thiamin-pyrophosphat-effect test) to determine the prevalence of thiamin deficiency in ALS/MND patients.

Results: We found thiamine deficiency in 28% of our ALS/MND patients. Among these, 14 patients had slight (41%) and 20 patients severe (59%) thiamine deficiency. Signs of acute Wernicke encephalopathy at autopy was seen in only 8.7 % of ALS cases autopsied.

Conclusion: The two ALS/MND autopsy cases with morphological signs of Wernicke encephalopathy reported here in detail and the subsequent analysis of thiamine deficiency in 122 ALS/MND patients strongly indicate a risk of ALS/MND patients to develop B1 deficiency probably triggering Wernicke encephalopathy in few end-stage ALS/MND patients.

Introduction

Thiamin (vitamin B1) deficiency is often related to malnutrition and causes severe neurological disorders, i.e. Wernicke encephalopathy and Beriberi disease [28]. Treatment with thiamine supplementation protects from those diseases and is used for therapy. Nevertheless, thiamine deficiency is underdiagnosed especially with regard to 1) iatrogenic causes such as use of diuretics [1] and chemotherapeutic agents [2], 2) long term parenteral application of carbohydrates without supplementation of thiamine, 3) gastrointestinal malabsorption (gastric banding, chronic gastrointestinal diseases), 4) reduced intake due to alcoholism [3], dementia [4], or diets [5], 5) hyperemesis gravidarum [6] and 6) increased catabolism, e.g. caused by neoplasias [2].

Thiamine storage in liver, heart, kidney and brain lasts for a few days to maximum 2-4 weeks in cases of deficient intake. Thiamin functions as a coenzyme for energy metabolism (citrate cycle, pentose 5-phosphate pathway). Accordingly, thiamine deficiency leads to catabolism with reduced synthesis of amino acids, lipids, neurotransmitters, collagen and to an intracerebral lactate acidosis caused by reduced glucose utilization, finally ending in a vicious circle of decreased absorption and metabolism of thiamine [7].

Amyotrophic lateral sclerosis (ALS/MND) is a prototype neurodegenerative disorder of the motor neuron disease group with degeneration of anterior horn cells in the spinal cord, motor nuclei of cranial nerves and pyramidal cells of the motor cortex resulting in progressive paresis of skeletal muscles. The amyotrophy typically begins focal and continuously involves more and more adjacent muscles. In the course of the disease, paresis of diaphragm and intercostal musculature leads to global

respiratory insufficiency. 25% of ALS/MND patients present with paresis-related dysphagia resulting in weight loss, malnutrition and cachexia[9].

The intention of this report is to highlight thiamine deficiency (probably causing Wernicke encephalopathy-related lesions) as a potential complication in the course of ALS/MND, which was unexpectedly found at autopsy in two (= 8.7 %) patients of a sample of 23 ALS/MND autopsy cases. To clarify its importance and to determine its prevalence, we additionally analyzed laboratory signs of thiamine deficiency in 122 ALS/MND patients seen in the University of Ulm Neurology Department.

Case 1

The patient attended our outpatient clinic reporting the following symptoms: dysarthria, dysphagia with pseudohypersalivation and an involuntary weight loss of 10 kilograms within five months. He observed spontaneous fasciculations and an unaccustomed breathlessness during physical stress. At an external hospital, probable ALS/MND was diagnosed. Treatment with riluzole was started and the patient was sent to the University of Ulm Neurology Department for second opinion.

Clinical exploration revealed a good physical status (body mass index 27.2) ??? months before death. The diagnosis of ALS/MND (for details see supplement 1 *material and methods*) was confirmed. In the course of the disease reevaluation of the clinical status was carried out to adjust the treatment. The patient developed generalized muscular atrophy and paresis requiring wheel chair use and leading to further weight loss (BMI 23.5). Percutaneous endoscopic gastrostomy (PEG) and non-invasive ventilation (NIV) were refused by the patient. Nutrition was limited to liquid alimentation. The patient received home care until death eleven months after his first visit. Informed consent was granted by the next of kin for an autopsy and scientific use of the tissue obtained at autopsy.

Neuropathological examination (performed according to German law) revealed a normal brain weight. The sole macroscopic abnormality was a reddish coloration of the corpora mamillaria and the periventricular thalamus. The spinal cord was macroscopically unremarkable.

Microscopically, multiple fresh hemorrhages were observed in the mammillary body and in the medial portion of the thalamus with the pattern of acute Wernicke encephalopathy (figure 1 a/b). Prussian blue staining did not detect siderophages (figure 1c). As such, there was no evidence for earlier bleedings into these regions. Fresh bleedings elsewhere in the brain indicating general agonal congestion-related bleedings were not found. A reduced number of spinal cord motor neurons was observed. Phospho-TDP43-(pTDP43) positive preinclusions in pyramidal neurons of the frontocentral (figure 1d) and parietal cortex and pTDP43-positive skein-like inclusions in few remaining ventral horn neurons of the thoracic spinal cord (figure 1e) were seen providing the neuropathological correlative for ALS/MND. The distribution of pTDP43 lesions represented a pattern related to ALS/MND stage 3 according to Brettschneider et al.[10]. Only brain and spinal cord autopsy was permitted precluding a cause of death statement.

Case 2

At primary clinical examination, the patient showed a spastic tetraparesis with enhanced tendon reflexes, beginning distal atrophies and generalized fasciculations. Cerebral and spinal MRI was normal. Electromyographically acute and chronic neurogenic changes were observed. Cerebrospinal fluid analysis showed mild signs of inflammation (for details see supplement 1 material and methods). However, treatment with methylprednisolone had no effect on the clinical status. Further

progression led to severe dysphagia, dysarthria and worsening of the spastic tetraparesis with generalized muscular atrophy. Symptoms and course of disease were consistent with the diagnosis of ALS/MND. Wheel chair use was required. Parenteral gastrostomy was refused. The patient died at home. Informed consent was granted by the next of kin for an autopsy and scientific use of the tissue obtained at autopsy.

The autopsy revealed fresh hemorrhages in the paraventricular nucleus and mild fresh hemorrhages in the mammillary body of the hypothalamus (figure 1 f/g) suggestive for acute Wernicke encephalopathy. The Prussian blue staining did not detect siderophages as signs of blood resorption (figure 1h). Fresh bleedings elsewhere in the brain indicating general agonal congestion-related bleedings were not found. pTDP43-positive preinclusions were found in pyramidal neurons of the frontocentral cortex (figure 1 i), pTDP43-positive skein-like inclusions occurred in few remaining neurons of the hypoglossal nucleus and in the inferior olivary nucleus (figure 1 j/k), confirming the diagnosis of ALS/MND. The distribution of pTDP43 lesions represented ALS/MND stage 3 according to Brettschneider et al.[10]. Only brain and spinal cord autopsy was permitted precluding a cause of death statement.

Material and Methods

Patients

We investigated a total of 122 ALS patients (age 62±13 years, 62 males, 60 females), among them 22 patients with pure bulbar form, 29 patients with pure spinal form of ALS and 71 patients with both spinal and bulbar symptoms (stated at time of involvement). 35 ALS patients were provided with a PEG. Patients were diagnosed according to the El-Escorial-criteria[11]. All patients or their relatives gave their informed consent prior to the study that was performed according to the ethical

standards of Helsinki. Votes of the University of Ulm ethics committee cover the clinical observations and the post-mortem analysis of donated autopsy tissue.

Diagnosis of thiamine-deficiency

Thiamine deficiency was calculated in a two-step procedure: basal activity of the erythrocyte Transketolase (TK, 2 ml EDTA blood, spectroscopy, reference range between 42 and 69 U/l, *Synlab* laboratory) and measurement of TK-activity after addition of coenzyme thiamine-pyrophosphat in vitro (= TPP-effect; two blood samples were obtained for each patient: one before stimulation with coenzyme thiamin-pyrophosphate, a second one ??? min. after stimulation; 2ml EDTA blood, photometry, *Synlab* laboratory). In case of thiamine deficiency, basal TK-activity rises after addition of TPP. The percentage of activation of TK by thiamine pyrophosphate is normal in the range of 0-15%; an increase of the TPP-effect >15% indicates mild and >20% severe thiamine deficiency[12,13].

Apart from thiamine deficiency, diabetes mellitus, liver diseases accompanied with elevated transaminases and medication with diuretics and antacida were also able to influence transketolase activity. For this reason, it was essential to combine measurement of transketolase activity with analysis of the enzyme-activity after addition of TPP to saturate the TK with thiamine leading to maximum functional capacity of the enzyme (TPP-effect).

A reference group was not studied because we focused on ALS/MND patients to determine their prevalence of thiamine deficiency and to estimate whether supplementation of thiamine is required. Patients attending a neurological hospital often belong to the well known risk groups such as elderly and/or demented persons, alcoholics, and patients with catabolism. Therefore, a control group of patients seen in a neurological hospital would represent a biased sample. Moreover, the thiamine

status in random samples of defined populations is well known from other studies [14-18]. Therefore, we refer to these studies to interpret the prevalence of thiamin deficiency found in our ALS/MND sample.

Statistical analyses

Statistical analyses were performed using the software *SigmaStat, 3.5*. Values of $p<0.05$ were set as statistical relevant. Comparison of groups was performed by t-test/ANOVA.

Results

We found a TPP-effect in 34 (28%) out of 122 ALS patients. Among these, 14 patients had mild (41%) and 20 patients severe (59%) thiamine deficiency as indicated by TPP-effect of 16-20% for mild thiamine deficiency and higher than 20% for severe thiamin deficiency. Interestingly, none of these patients showed symptoms of Wernicke encephalopathy or Beriberi disease. As chronic alcohol abuse is a possible risk factor for thiamine deficiency, we screened our patient files for information about alcohol intake and laboratory liver parameters (Alanin-Aminotransferase, Aspartat-Aminotransferase, Gamma-Glutamyl-Transferase, mean corpuscular volume). Only ALS/MND patients without a history of alcoholism and without laboratory signs of liver dysfunction were considered for assessing the prevalence of thiamine-deficiency in this study.

As patients with primarily bulbar symptoms are predestined for malnourishment, we anticipated higher prevalence of thiamine deficits in this ALS subgroup than in those patients presenting mainly with myatrophies of the limbs. Contrary to our expectations, this was not the case ($p=0.46$). In the subgroup of the bulbar (18%)

versus spinal form (21%) of ALS, no relation was found between thiamine deficiency and the severity of ALS symptoms (p=0.48), graduated by the ALS/MND-functional rating score ALS-FRS-R[19]. Out of the PEG-provided patients, 25% had thiamine deficiency as well; comparison between PEG-provided patients with dysphagia and patients with dysphagia without PEG showed no statistical significance in development of thiamine deficiency (p=0.97).

Discussion

Here, we report morphological signs of acute Wernicke encephalopathy in two patients with ALS/MND, approved by autopsy. Among our ALS postmortem examinations (n=23), we detected Wernicke encephalopathy-indicative abnormalities in only two out of 23 (8.7%) ALS/MND cases. In spite of dysphagia, both patients refused parenteral gastrostomy. Both of them died at home, precluding clinical investigation shortly before death to identify symptoms of Wernicke encephalopathy. Home care also explains the missing TK and TPP-effect data for both of the patients.

However, diagnosis and herewith combined prevalence of Wernicke encephalopathy has been gleaned mostly from retrospective autopsy studies and the latter is probably underestimated at 2%[20]. Only a fraction of cases (approximately 15%) are diagnosed antemortem and represent cases with the typical clinical signs of Wernicke encephalopathy[21]. Except for alcohol abuse cases, Wernicke encephalopathy is usually not recognized before autopsy.

Importantly, the classical clinical triad of Wernicke encephalopathy (mental status changes, ophthalmoplegia and gait ataxia) is only present in ~ 10% of cases[20]. Moreover, ophthalmoplegia remains only one of numerous ocular findings that include nystagmus, sixth nerve palsy, ptosis, retinal hemorrhage, papilloedema, and

(less frequently) anisocoria or miosis[27]. Moreover, neither diagnostic tool (MRI, blood levels) can confirm the diagnosis of Wernicke encephalopathy on its own [27].

Our finding that 28% of the ALS/MND patients admitted to the University of Ulm Neurology department exhibit laboratory signs of thiamine deficiency without clinical symptoms indicates that ALS/MND patients are at risk for developing Wernicke encephalopathy. A possible explanation may be malnutrition due to dysphagia and waiving parenteral gastrostomy as in the two cases reported here. However, as there was no different prevalence between the bulbar and the spinal form of ALS/MND and no relation of thiamine deficiency with the severity of respective ALS/MND symptoms, our data suggest in agreement with the literature (Zitate!!!) no existing reliable predictor for the development of thiamine deficiency.

Interestingly, Meiniger et al. (Zitation fehlt!) investigated a large cohort of ALS/MND patients postmortem and found pulmonary insufficiency due to pneumonia to be the most frequent cause of death in their samples. The second cause of death was sudden death that was hypothezised to be a consequence of autonomic dysfunction. As Wernicke encephalopathy neuropathologically affects the hypothalamus and is consecutively able to lead to autonomic (particularly cardial) dysfunctions that are known to be frequent in ALS patients, a connection between these two pathomechanisms may be plausible.

As a clinical consequence of our finding of thiamine-deficiency in 28 % of ALS/MND patients it appears to be recommendable for clinical practice to pay attention to those patients being part of the well known risk groups to develop Wernicke encephalopathy now including also ALS/MND patients that are listed in table 1.

Table 1: Risk factors for the development of thiamine deficiency either caused by reduced intake or by diminished absorption.

risk factor	**Reference**
alcohol abuse	[2]
hyperemesis gravidarum	[6]
Diarrhea	[22]
gastrointestinal diseases with malabsorption	[23]
gastrointestinal surgery	[24]
cancer diseases	[2]
anorexia nervosa / diets	[5]
specific medication (diuretics and chemotherapeutics)	[1,2]
magnesium depletion	[25]
carbohydrate intake / parenteral nutrition	[26]
elderly people (dementia)	[4]
ALS/MND	this study

In a second step, the clinician should be aware to symptoms of thiamine deficiency (that can be absent as stated above). Both aspects – the affiliation to a risk group as well as potential clinical symptoms - should lead to assess thiamine levels at a low threshold. Patients suffering from chronic progressive diseases like ALS/MND should be informed about thiamine deficiency when diagnosed as a potential complication and about its clinical manifestations that can present beyond Wernicke encephalopathy (polyneuropathy, high-output congestive heart failure with and without edema, gastrointestinal discomfort like vomiting, abdominal pain, lactate acidosis, and Marchiafava-Bignami syndrome)[28]. Additionally, the possible fatal outcome ought to be discussed so that the ALS/MND-patient can decide in favour or against supplementation (for application schema, see table 2).

Table 2: EFNS guidelines for diagnosis, therapy and prevention of Wernicke encephalopathy[29].

Abbreviations: WE = Wernicke encephalopathy; GPP = good practice point.

1.) The clinical diagnosis of WE should take into account the different presentations of clinical signs between alcoholics and non alcoholics (Recommendation Level C); although prevalence is higher in alcoholics, WE should be suspected in all clinical conditions which could lead to thiamine deficiency (good practice point – GPP).
2.) The clinical diagnosis of WE in alcoholics requires two of the following four signs (Level B):

(i) dietary deficiencies (ii) eye signs (iii) cerebellar dysfunction [12] either an altered mental state or mild memory impairment
3.) Thiamine in blood sample should be measured immediately before its administration (GPP).
4.) MRI should be used to support the diagnosis of acute WE both in alcoholics and non alcoholics (Level B).
5.) Thiamine is indicated for the treatment of suspected or manifest WE. It should be given before any carbohydrate, 200 mg thrice daily, preferably intravenously (Level C).
6.) The overall safety of thiamine is very good (Level B).
7.) After bariatric surgery, follow-up of thiamine status for at least 6 months (Level B) and parenteral thiamine supplementation (GPP) is recommended.
8.) Parenteral thiamine should be given to all at-risk subjects admitted to the Emergency Room (GPP).
9.) Patients dying from symptoms suggesting WE should have an autopsy (GPP).

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Competing interest

SJ and ACL have no potential conflict of interest, including relevant financial interests, activities, relationships, and affiliations.

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Figure legends

Figure 1 a, b: Multiple fresh hemorrhages in the mammillary body of the case 1 hypothalamus exhibiting the pattern of Wernicke encephalopathy. **c**: The prussian blue staining did not detect siderophages as signs of resorption. **d**: Phospho-TDP43-(pTDP43) positive preinclusions were found in pyramidal neurons of the frontocentral cortex. **e**: pTDP43-positive skein-like inclusions occurred in few remaining ventral horn neurons of the thoracic spinal cord confirming the clinical diagnosis of amyotrophic lateral sclerosis. **f, g**: Fresh hemorrhages in the paraventricular nucleus and single fresh hemorrhages in the mammillary body of case 2 hypothalamus. **h**: The prussian blue staining did not

detect siderophages as signs of resorption. **i**: pTDP43-positive preinclusions were found in pyramidal neurons of the frontocentral cortex. **j, k**: pTDP43-positive skein-like inclusions occurred in few remaining neurons of the hypoglosseal nucleus and in the inferior olivary nucleus confirming the clinical diagnosis of amyotrophic lateral sclerosis.

Stainings: **a, b, f, g**: hematoxilin & eosin; **c, h**: Prussian blue; **d, e, l, j, k**: Immunohistochemistry with antibodies directed against pTDP43 (pS409/410-2, Cosmo Bio Co., Ltd, Tokyo, Japan, 1/10000, microwave pretreatment). Visualization by using biotinylated secondary antibodies, ABC-complex and diaminobecindine-HCl (Vector, Burlingame, USA).

Calibration bar in **k** corresponds to: **a, f**: 800µm; **b, c**: 160µm; **d, e, g-k**: 15µm.

Jesse et al.
Fig. 1